**BINDING AND LOCALIZATION OF RECOMBINANT LUBRICIN TO ARTICULAR CARTILAGE SURFACES**

*Jones, ARC; **Gleghorn, JP; §Hughes, CE; *Fitz, LJ; *Zollner, R; §Wainwright, SD; §Caterson, B; *Morris, EA; **Bonassar, LJ; ++Flannery, CR
++Wyeth Research, Cambridge, MA
cflannery@wyeth.com

**INTRODUCTION**

Lubricin is a secreted, cytoprotective glycoprotein which contributes to the essential boundary lubrication mechanisms necessary for maintaining low friction levels at articular cartilage surfaces. Lubricin occurs as a soluble component of synovial fluid, and is synthesized and localized in the superficial layer of articular cartilage; however the molecular interactions responsible for lubricin localization at this site are not well understood. In the current studies, we used non-denaturing extraction conditions to dissociate endogenous lubricin from the superficial zone of cartilage explants, and established that recombinant full-length lubricin, and a protein construct comprising the C-terminal (hemopexin-like) domain of lubricin (“LUB-C”) are able to specifically and effectively bind to the articular surface. In contrast, the N-terminal region of lubricin (construct “LUB-N”) exhibited no appreciable cartilage-binding ability, but displayed the capacity to dimerize, and thus potentially influence lubricin aggregation.

**METHODS**

Recombinant full-length lubricin was expressed in CHO cells and purified from conditioned media by sequential heparin-affinity and anion exchange chromatography. N-terminally FLAG-tagged LUB-C (exons 7-12) and LUB-N (exons 2-5) constructs were expressed in *Drosophila* S2 cells (Invitrogen) and purified using heparin- and FLAG M2-affinity columns. Western immunoblot analysis and/or immunohistochemistry (IHC) was performed using anti-lubricin antibodies (6-A-1, 3-A-4, G35, 06A10) or anti-FLAG M2 antibodies. Bovine articular cartilage discs were extracted for 20 min at 4°C in PBS containing 1.5M NaCl to remove endogenous lubricin. Formalin-fixed cartilage discs were embedded in paraffin and microtome-sectioned at 5-micron intervals. For binding experiments, sagittal sections of extracted discs were incubated for 1h at 18°C with synovial fluid or recombinant lubricin preparations.

**RESULTS**

Endogenous lubricin was efficiently dissociated from cartilage surfaces by extraction with PBS containing 1.5M NaCl (Fig. 1); no additional lubricin was removed by subsequent extraction with 4M guanidine HCl. Addition of synovial fluid to extracted cartilage resulted in specific binding of lubricin to the superficial zone (Fig. 2A), and similar results were observed for recombinant full-length lubricin and a C-terminal domain construct (LUB-C) (Figs. 2B and 3). Disruption of lubricin protein secondary structure, by reduction and alkylation of disulfide bonds, substantially diminished such binding (Fig. 2B), demonstrating a requirement for protein secondary structure in facilitating relevant lubricin localization at tissue interfaces. Recombinant LUB-N, representing the N-terminal region of lubricin displayed little to no cartilage-binding affinity, however this protein construct exhibited spontaneous dimerization, indicating that N-terminal domain interactions may facilitate the aggregation of lubricin monomers.

**DISCUSSION**

Palliative options for structural modification during joint diseases such as osteoarthritis (OA) are currently limiting. Dysregulated cartilage proteoglycan and collagen turnover is an OA hallmark, and degenerative changes at cartilage surfaces, including lubricin diminution, emblazon early pathophysiology. Disease-associated catabolic processing of lubricin may thus also be envisioned, and it is evident from the current study that even limited degradation affecting terminal protein domains could profoundly impact lubricin functionality. Enhancing cartilage endurance, for example via application of functionally competent biolubricant formulations, would rationally represent an efficacious means to help alleviate OA disease progression.

**REFERENCES:**


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**AFFILIATED INSTITUTIONS FOR CO-AUTHORS:**

**Cornell University, Ithaca, NY; §Cardiff University, Cardiff, UK**